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Sonication as a Diagnostic Approach Used to Investigate the Infectious Etiology of Prosthetic Hip Joint Loosening

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Abstract

The purpose of the study was to evaluate the usefulness of sonication for the diagnosis of prosthetic joint infections (PJIs) by its comparison with periprosthetic tissues (PTs) and synovial fluid (SV-F) cultures. The study groups included 54 patients undergoing exchange of total hip prostheses for so called "aseptic" loosening occurring without clinical manifestations of an accompanying PJI and 22 patients who developed a sinus tract communicating with the prosthesis which was indicative of an ongoing infectious process. Significant positive culture results were obtained among 10 (18.5%) patients with "aseptic" implant failure and in 18 (81.8%) patients who developed a sinus tract. Sonicate-fluid (S-F) yielded bacterial growth in all culture-positive patients with "aseptic" loosening vs. 15 patients with presumed PJIs. There was a concordance in terms of bacterial species isolated from S-F and conventional cultures from individual patients. Coagulase-negative staphylococci were isolated most frequently. Sensitivity of sonication (75%) exceeded that estimated for PTs (69%) and SV-F (45%) cultures. We conclude that identification of causative agents of PJIs which is critical to further therapeutic decisions is aided by the combination of sonication and conventional culture.

Key words: sonication, periprosthetic infections, coagulase-negative staphylococci

Introduction

Joint replacement surgery is the major procedure used for the treatment of degenerative joint diseases (osteoarthritis) and bone fractures (Trampuz and Zimmerli 2005; Achermann *et al.*, 2010). Less than 10% of prosthesis recipients develop implant-associated complications during their lifetime, predominantly diagnosed as aseptic failure. Infections associated with prosthetic joints occur less frequently than aseptic implant dysfunction, but represent the most devastating complication with high morbidity and substantial cost (Trampuz and Zimmerli 2005; Trampuz *et al.*, 2006; Trampuz *et al.*, 2007; Monsen *et al.*, 2009).

Diagnosis of prosthetic joint infections (PJIs) remains a challenge, as clinical signs and a laboratory investigation, including microbiological findings do not always distinguish aseptic loosening from implant dysfunction associated with an ongoing infectious process (Esteban *et al.*, 2008; Monsen *et al.*, 2009). Since treatment strategies are fundamentally different, it is crucial to accurately distinguish these two clinical entities (Ince *et al.*, 2004; Trampuz *et al.*, 2006; Trampuz *et al.*, 2007).

Currently, cultures of synovial fluid and intraoperative periprosthetic tissue represent the standard method for diagnosing PJIs (Trampuz *et al.*, 2006; Trampuz *et al.*, 2007; Esteban *et al.*, 2008; Achermann *et al.*, 2010). However, the pathogenesis of biomaterial-related infections is primarily associated with the formation of microbial biofilm where bacteria change their phenotypes to an extremely resistant sessile form of life (Trampuz *et al.*, 2007; Esteban *et al.*, 2008). In these structures bacteria live clustered together in a highly hydrated extracellular matrix attached to a surface where they are protected from host defence cells and antibiotics (Nelson *et al.*, 2005). Moreover, depletion of metabolic substances and accumulation of waste products in the biofilm structure causes microbes to enter a slow- or non-growing (stationary) state in which they are less susceptible to growth-dependent antimicrobial killing (Trampuz *et al.*, 2003; Trampuz and Zimmerli 2005; Esposito and Leone 2008). Subclinical infection

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caused by such persistent, although relatively dormant, bacteria may interfere with functioning of the prosthesis and lead to the loosening in the absence of overt manifestations of infection. Therefore, it has been assumed that a portion of joint implant failures clinically attributed to aseptic loosening may in fact be the result of oligosymptomatic, low-grade infections caused by bacteria existing in the form of biofilm (Nelson *et al.*, 2005).

Other important concepts which may give rise to diagnostic problems associated with PJIs include the failure to recognize bacterial small colony variants (SCVs) induced during growth *in vivo* and the presence of microorganisms inside host cells such as osteoblasts (Nelson *et al.*, 2005; Monsen *et al.*, 2009). These microbial strategies significantly hamper the diagnostic yield of conventional cultures which fail to detect biofilm-embedded bacteria or bacteria residing inside eukaryotic cells (Nelson *et al.*, 2005; Esteban *et al.*, 2008).

Sonication appears to be the most promising method among the newer techniques used for the diagnosis of infected implants since it disrupts the bacterial biofilm layer preserving microbial viability (Nelson *et al.*, 2005; Trampuz *et al.*, 2007; Monsen *et al.*, 2009).

Herein, we compared culture of samples obtained by sonication of explanted hip prostheses with conventional culture of periprosthetic tissues and synovial fluid samples for the microbiological diagnosis of PJI among patients undergoing revision due to implant loosening. The purpose of the study was to evaluate the usefulness of sonication in order to increase the efficacy of microbiological culture in the diagnosis of PJIs.

Experimental

Materials and Methods

Sample collection The study enrolled 76 patients undergoing surgical revision of total hip prostheses. The patients were categorized into two groups:

- 54 patients (mean age: 72.3 ± 10.3 years; mean time to the onset of the loosening symptoms: 79 ± 61.3 months) in whom prosthesis failure occurred without accompanying clinical manifestations of an ongoing periprosthetic infection.

Due to the lack of clinical manifestations of PJI such as a sinus tract and/or purulence in the affected joint, the patients were initially qualified as suffering from "aseptic" implant loosening.

- 22 patients (mean age: 67.5 ± 10.9 years; mean time to the onset of the loosening symptoms: 40.9 ± 38.2 months) who developed a sinus tract communicating with the prosthesis which was indicative of an ongoing PJI.

Intraoperatively, tissue samples from the close proximity of the implant and demonstrating the most obvious inflammatory changes were collected for microbiological studies. At least three tissue samples were collected from each patient. The synovial fluid was collected intraoperatively from patients with the clinical diagnosis of aseptic loosening for leukocyte count and differential as well as for microbiological culture. The explanted prosthetic components were placed in 1-liter, straight-sided, wide-mouthed polypropylene jars that had been autoclaved at 132°C for 15 minutes. The specimens were processed by the microbiology laboratory within 2 hours.

Conventional microbiological methods. Synovial fluid was inoculated in 100 µl aliquots onto a set of routine aerobic and anaerobic bacteriologic media. The plates were incubated at 35–37°C for up to 14 days. Tissue specimens were inoculated into thioglycollate broth and incubated at 35–37°C. Cloudy thioglycollate broth was subcultured onto conventional bacteriologic media.

Sonication of removed prostheses. Five hundred milliliters of sterile saline were added to each container. The container was vortexed for 30 seconds and subsequently subjected to sonication (Bransonic® Ultrasonic Cleaner G.HEINEMANN) for 7 minutes at the temperature of 20°C. Sonication was followed by additional vortexing for 30 seconds. The resulting sonicate fluid was removed under aseptic conditions and placed into 50-ml sterile Falcon tubes. Samples were then centrifuged at 4000 rpm for 20 minutes. 100 µl aliquots of the sedimented sonicate fluid were inoculated onto a set of routine aerobic and anaerobic bacteriologic media. Incubation (at 35–37°C) lasted for up to 14 days. The sonication procedure was based on the publications of Trampuz *et al.* (2007) and Monsen *et al.* (2009).

Identification of cultured microorganisms All media were inspected daily for microbial growth. The culture result was considered positive if there were at least 5 colony-forming units of the same organism on either plate. Isolated microorganisms were identified to the species level using commercially available biochemical tests (API Staph, Api 20E, Api 20A, Api 20 Strep; BioMérieux, France).

Definition of PJIs – interpretation of positive culture results. In the group of 54 patients prosthesis failure occurred in the absence of clinical manifestations of PJI such as a sinus tract and/or purulence in the affected joint. The microbiological diagnostic proceedings were supplemented by analysis of local (synovial fluid leukocyte count and differential) and systemic markers of inflammation (erythrocyte sedimentation rate/ESR, C-reactive protein/CRP) in order to verify the clinical significance of positive culture results and to minimize the risk of interpretation of false-positive culture results.

Table I
Criteria used for the diagnosis of prosthetic joint infection according to Parvizi *et al.* (2011)

Sinus tract communicating with the prosthesis, **or**	
Isolation of a pathogen by culture from 2 or more separate tissue or fluid samples obtained from the affected joint, **or**	
Existence of 4 of the following 6 criteria:	elevated ESR (> 30 mm/h) or serum CRP (> 10 mg/l) concentration*
	elevated (> 1700 cells/μl) synovial leukocyte count*
	elevated (> 65%) synovial neutrophil percentage*
	presence of purulence in the affected joint
	isolation of a microorganism in one culture of periprosthetic tissue or fluid
	greater than 5 neutrophils per high-power field in 5 high-power fields observed from histologic analysis of periprosthetic tissue at × 400 magnification**

* – cut-off values adopted from Trampuz *et al.* (2007); ** – pathohistological examination was not performed in the study.
The levels of systemic markers of inflammation were determined among all patients in the direct preoperative period; the synovial fluid leukocyte count and differential were assessed only among patients with the clinical diagnosis of "aseptic" loosening.

The clinical diagnosis of PJI associated with implant loosening in the consecutive group of 22 patients was made on the basis of the development of a sinus tract communicating with the prosthesis. It is a definitive symptom of an ongoing periprosthetic infection according to recommendations published by Parvizi *et al.* (2011). The microbiological diagnostic proceedings were supplemented by the analysis of systemic markers of inflammation (ESR, CRP) in order to verify the infectious character of the implant failure and to interpret the clinical significance of positive culture results. Table I summarizes the criteria of Parvizi *et al.* (2011) which have been applied for the diagnosis of PJIs among patients enrolled in the study.

Sensitivity and specificity of the applied diagnostic methods. Calculations used to describe sensitivity and specificity of the applied diagnostic procedures (sonicate-fluid culture, periprosthetic tissue culture, synovial fluid culture, serologic tests, synovial fluid leukocyte count and differential) were as follows:

Sensitivity = true positives/(true positives + + false negatives)

Specificity = true negatives/(true negatives + + false positives)

Results

Culture results in patients with implant loosening occurring without accompanying clinical manifestations of infection ("aseptic" loosening). In the group of 54 patients who did not develop clinical manifestations of PJI, positive culture results were obtained among 12 (22%) patients. Sonicate was the most common material from which bacteria were isolated – microbial growth was reported for 11 out of the 12 patients. Positive culture results in the synovial fluid and/or periprosthetic tissue fragments (with or without accompanying positive sonicate-fluid culture results) were obtained among 6 patients. Sonicate was the only material from which bacterial isolates were cultivated in 3 patients (Table II, patients: A4-A42).

Staphylococci were cultivated the most frequently. *Staphylococcus epidermidis* was the predominant species as it was cultured from 7 patients. Other staphylococcal species included *Staphylococcus warneri* (2 patients) and *Staphylococcus aureus* (1 patient). Growth of other bacteria such as single strains of *Enterobacter cloacae*, *Streptococcus mitis,* and *Propionibacterium acnes* was also reported. A mixture of two different bacterial species (*S. epidermidis* and *P. acnes*) was cultured in the sonicate fluid obtained from one patient (no. A40). Tissue and synovial fluid cultures obtained from this patient revealed the growth of *S. epidermidis* only.

Culture results in patients with prosthesis failure accompanied by the development of a sinus tract (septic loosening). Positive results of microbial cultivation were obtained among 18 (81.8%) out of the 22 patients. Tissues were the most common material form which bacteria were cultured – 17 of the 18 patients were positive for microbial growth in this material. Bacterial growth was observed in the sonicate fluid obtained from 15 patients. Positive culture results in the sonicate fluid were accompanied by bacterial growth in the periprosthetic tissues among 14 of the 15 patients. In the case of 3 patients only the periprosthetic tissue specimens yielded bacterial growth (Table II).

Staphylococci represented the only group of cultivated microorganisms. *S. epidermidis* – the predominant species – was isolated from 11 patients. *S. aureus* was isolated from 3 patients. Other isolates included: single isolates of *Staphylococcus lugdunensis, Staphylococcus cohnii*,

Table II
The levels of inflammatory mediators and microbiological culture results in patients enrolled in the study

No. of patient n = 12 (age/gender)	ESR (mm/h)	CRP (mg/l)	Synovial fluid leukocyte count and differential		Type of specimen subjected to culture			Microorganism cultured
			Leukocytes (cells/μl)	Neutrophils (%)	S-F	SVF	PTs (no.)	
A4 (62/F)	**88**	6.3	**12890**	**99**	+	–	+ (1)	*Staphylococcus epidermidis*
A6 (81/F)	**39**	0.8	**5130**	**72**	+	–	–	*Staphylococcus warneri*
A11 (74/F)	**52**	**36.67**	**6503**	**95**	+	–	+ (2)	*Enterobacter cloacae*
A17 (81/F)	27	1.2	**7069**	**97**	+	+	–	*Staphylococcus epidermidis*
A19 (61/F)	**42**	**36.76**	**2606**	**94**	+	+	+ (4)	*Staphylococcus epidermidis*
A26 (75/F)	**49**	**28.09**	1289	**94**	+	+	–	*Staphylococcus epidermidis*
A31 (68/F)	13	**13.29**	**149600**	**95**	+	+	+ (3)	*Staphylococcus aureus*
A40 (50/F)	19	0.74	**37600**	**97**	+	+	+ (1)	*Staphylococcus epidermidis* *Propionibacterium acnes* (S-F culture)
A56 (70/F)	**91**	**72.99**	**54860**	**95**	+	–	–	*Streptococus mitis*
A57 (71/F)	**52**	**11.89**	**7000**	**81**	+	–	+ (2)	*Staphylococcus epidermidis*
A3*	14	1.02	**8470**	47	–	+	–	*Staphylococcus warneri*
A42*	**38**	**14.3**	240	23	+	–	–	*Staphylococcus epidermidis*
S1 (61/M)	**50**	**11.9**			+		+ (3)	*Staphylococcus epidermidis*
S2 (39/M)	**66**	**29.5**			+		+ (2)	*Staphylococcus epidermidis*
S3 (59/M)	**50**	1.6			–		+ (1)	*Staphylococcus epidermidis*
S4 (70/M)	**76**	**51.4**			+		+ (2)	*Staphylococcus aureus*
S5 (65/M)	**46**	**19.5**			–		+ (2)	*Staphylococcus epidermidis*
S6 (76/M)	**58**	**40.6**			+		+ (3)	*Staphylococcus aureus*
S7 (60/M)	**64**	**29.8**			+		+ (3)	*Staphylococcus epidermidis*
S8 (78/M)	28	2.7			+		+ (3)	*Staphylococcus epidermidis*
S10 (75/M)	**46**	6.8	ND		+	ND	+ (2)	*Staphylococcus simulans* (S-F culture) *Staphylococcus capitis* (PT culture)
S11 (84/F)	26	3.6			+		+ (1)	*Staphylococcus epidermidis*
S14 (65/F)	**60**	**11.7**			–		+ (2)	*Staphylococcus epidermidis*
S15 (58/M)	**65**	**35.8**			+		+ (3)	*Staphylococcus aureus*
S16 (69/F)	**60**	7.3			+		+ (3)	*Staphylococcus warneri*
S19 (71/F)	**74**	**93**			+		+ (3)	*Staphylococcus epidermidis*
S20 (65/M)	20	4.6			+		+ (2)	*Staphylococcus lugdunensis*
S21 (78/F)	**76**	**68.5**			+		+ (3)	*Staphylococcus epidermidis*
S22 (75/F)	**60**	**15.3**			+		+ (1)	*Staphylococcus epidermidis*
S23 (84/M)	22	0.8			+		–	*Staphylococcus cohnii*

A4-A42 – patients with clinically "aseptic loosening"; S1-S23 – patients with prosthesis failure accompanied by the development of a sinus tract (septic loosening); F – female; M – male; ND – not done; S-F – sonicate fluid; SVF – synovial fluid; PTs – periprosthetic tissues; +/–: positive/negative culture result;
* – interpreted as contamination – see section of results entitled: "Interpretation of the clinical significance of positive culture results"; boldface was used to highlight values above cut-off

S. warneri, as well as *Staphylococcus simulans* and *Staphylococcus capitis* (cultured from one patient). The latter two species grew in two different types of clinical samples obtained from the patient. Namely, *S. simulans* was identified in the sonicate-fluid culture whereas *S. capitis* was isolated from periprosthetic tissue cultures (Table II).

Analysis of the levels of local and systemic inflammatory markers among culture-positive patients. Among the 12 culture-positive patients in whom prosthesis loosening was not accompanied by the development of manifestations of PJI ("aseptic" loosening) the synovial fluid leukocyte count and the neutrophil percentage exceeded the cut-off values in 9 cases. Among 7 of these patients the elevated levels of local inflammatory mediators were also accompanied by high ESR and/or serum CRP concentration (Table II).

Among patients with the clinical diagnosis of septic loosening the synovial fluid was not available for cyto-

logical analysis. Only the preoperative levels of systemic inflammatory mediators were evaluated (Table II). Unexpectedly, 3 patients in this group (no. S8, S11, and S20) had normal levels of ESR and CRP in spite of the isolation of bacteria from both the sonicate-fluid samples and the periprosthetic tissues. Another patient (S23) with the parameters of systemic inflammatory mediators remaining within the normal range had a positive culture result from the sonicate fluid only; due to the development of the sinus tract communicating with the prosthesis the culture result was interpreted as significant. Among the remaining patients both ESR and CRP levels were elevated or only ESR values exceeded the cut-off (Table II).

Interpretation of the clinical significance of positive culture results. Following recommendations of Parvizi *et al.* (2011) PJI was diagnosed among 10 out of 12 culture-positive patients whose implant failure occurred without clinical manifestations of infection ("aseptic" loosening). We assume that in the remaining two patients (no. A3 and A42) culture results were false-positive and associated with contamination. This assumption was based on the fact that the positive culture results were obtained from single clinical specimens (synovial fluid and sonicate fluid, respectively). The positive culture result in the patient no. A3 was accompanied neither by elevated ESR/CRP concentration nor by a differential of > 65% neutrophils in the synovial fluid. High synovial fluid leukocyte count (8470 cells/μl), in turn, might have been associated with rheumatoid arthritis which was a co-morbidity identified in the patient. In the patient no. 42, in turn, elevated ESR and CRP concentration were accompanied by a low leukocyte count and by a differential below 65% of neutrophils in the synovial fluid (Table II).

One false-negative culture result was assumed in a patient with the clinical diagnosis of "aseptic" loosening. The patient had high ESR (44 mm/h) and CRP level (40.25 mg/l) as well as high leukocyte count (3300 cells/μl) and the percentage of neutrophils (67%) in the synovial fluid. The above mentioned levels of nonspecific mediators of inflammation in serum could have been elevated in this patient due to the recent surgery (the symptoms of loosening developed within the first month after implantation); however, high synovial fluid leukocyte count and differential led to the formulation of conjecture about the infectious etiology of the implant failure.

Hence, it was concluded that infection was the most probable cause of implant dysfunction in 11 (20%) out of 54 patients with the clinical diagnosis of aseptic loosening.

In the group of 22 patients whose implant loosening was clinically diagnosed as septic due to the development of a sinus tract all positive culture results were considered significant. Four (18%) negative culture results were interpreted as false-negative.

Table III
Sensitivities and specificities of the applied diagnostic methods

Diagnostic method	Sensitivity (%)	Specificity (%)
Sonicate-fluid culture	75	97
Periprosthetic tissue culture	69	100
Synovial fluid culture	45	97
Synovial fluid leukocyte count	90	97
Synovial fluid neutrophil percentage	100	95
ESR	75	70
CRP concentration	62	80

Sensitivity and specificity of the applied diagnostic methods. Among the microbiological culture-based methods sonication was characterized by the highest sensitivity (75%) and a satisfactory specificity (97%). Periprosthetic tissue culture, in turn, was associated with an excellent (100%) specificity (Table III).

Analysis of the synovial fluid leukocyte count and differential was characterized by the highest sensitivity (90% and 100%, respectively) and high specificity (97% and 95%, respectively) – in this regard local inflammatory markers outweighed the diagnostic value of the serum inflammatory parameters such as ESR and CRP (see Table III for details).

Discussion

It has been reported that bacteria have the capacity to tightly adhere to artificial surfaces implanted in the human body. The pathogenesis of PJIs may explain the diagnostic problems as the bacteria associated with the implant surface cannot be removed by conventional microbiological methods, which are most often limited to aspiration of joint fluid or a swab of the implant and adjacent tissue, or both. Furthermore, bacteria are able to adopt a lifestyle that precludes their detection by conventional cultural methods, which is exemplified by the formation of a biofilm (Esteban *et al.*, 2008; Smeltzer *et al.*, 2009). Taking into account the above mentioned assumptions of the pathogenesis of biomaterial-related infections, considerable effort has been directed toward developing alternative approaches of bacterial recovery such as sonication of explanted devices followed by cultural analysis of the resultant fluid (Gristina and Costerton 1985; Dobbins *et al.*, 1998; Tunney *et al.*, 1998; Tunney *et al.*, 1999; Esteban *et al.*, 2008; Smeltzer *et al.*, 2009; Holinka *et al.*, 2011).

Esteban *et al.* (2008) found sonication as an important tool increasing the chance of the diagnosis of device-related orthopaedic infections due to its greater

sensitivity in comparison with routine cultivation techniques. The sensitivity of sonication was determined as 94.1% and exceeded that of conventional cultures (88.2%). The specificity of sonication (42.8%), however, remained below that reported for the conventional cultures (100%). The authors revealed one case of contamination and six cases of unexpected positive cultures.

Higher sensitivity of sonication in comparison with conventional periprosthetic-tissue culture (78.5% vs. 60.8%, respectively) was also reported by Trampuz *et al.* (2007). The authors emphasized that 14 cases of PJI were detected by sonicate-fluid culture but not by prosthetic-tissue culture.

In the study reported here sonication has also been found a valuable approach to the diagnosis of PJI. The sensitivity of the sonicate-fluid culture (75%) exceeded that of periprosthetic tissue and the synovial fluid cultures (69% and 45%, respectively). Its specificity (97%), however, was below that determined for tissue cultures (100%) which was similar to observations reported by Esteban *et al.* (2008). In general, positive results of microbial cultivation were obtained in 12 (22%) out of 54 patients with the clinical diagnosis of aseptic failure. However, we assume that true PJI was associated with implant dysfunction in 11 (20%) patients since two false-positive and presumably one false-negative culture result were reported.

We consider the sonicate fluid a material of an important diagnostic value. Bacterial growth in this material was observed in all patients who suffered from subclinical infection, initially classified as aseptic implant loosening. Furthermore, the growth of microorganisms involved in the pathogenesis of two cases of PJI was achieved only in the sonicate-fluid culture, not in the conventional cultures of periprosthetic tissues or synovial fluid. The results support the hypothesis that sonication increases the chance of isolation of bacteria involved in the pathogenesis of implant failure especially in patients who do not develop overt manifestations of infection. They also indicate that in case of "aseptic" loosening microorganisms may be preferentially associated with the surface of prosthetic material whereas their concentration in periprosthetic tissues and synovial fluid may not be sufficient for their detection with the use of routine cultivation techniques.

The overwhelming majority of patients with the clinical diagnosis of PJI, in turn, had positive culture results both in the sonicate and periprosthetic tissue cultures. Moreover, one case of PJI would have been reported as culture-negative if sonication had not been applied.

Another appreciable advantage of the sonicate-fluid cultures is improved detection of polymicrobial PJIs (Trampuz *et al.*, 2007; Esteban *et al.*, 2008; Holinka *et al.*, 2011).

Esteban *et al.*, (2008) reported the isolation of more than one microorganism in the sonicate culture obtained from four patients with the clinical diagnosis of prosthetic hip joint infection. We have observed one polymicrobial infection in a patient with the clinical diagnosis of "aseptic" failure – the sonicate-fluid culture revealed the growth of *S. epidermidis* and *P. acnes*. The cultures of synovial fluid and periprosthetic tissues obtained from the patient revealed the growth of *S. epidermidis* only. The growth of *P. acnes* was detected after 11 days of incubation in strictly anaerobic conditions which supports the need for prolonged incubation of clinical materials collected from patients with suspicion of PJI which was highlighted by Schäfer *et al.* (2008) and Butler-Wu *et al.* (2011). The pathogenic potential of *P. acnes* as a low-virulence microorganism considered a commensal bacterium of the deep layers of skin, respiratory, divestive and eye mucosa has been a matter of discussion within the last years. There has been an increasing number of reports indicative of its role in the etiology of a variety of infections including PJIs (Zeller *et al.*, 2007; Zappe *et al.*, 2008; Kanafani *et al.*, 2009; Butler-Wu *et al.*, 2011). Similarly to other etiologic agents of PJIs, this bacterium has the propensity to form biofilms on orthopaedic biomaterials, thereby making eradication of an established infection difficult (Kanafani *et al.*, 2009). Therefore, sonication holds a promise as a technique able to increase the diagnostic yield. We assume that the culture result yielding *P. acnes* and *S. epidermidis* was clinically significant since the patient had high leukocyte count (37 600 cells/μl) and the neutrophil percentage (97%) in the synovial fluid. The symptoms of the loosening became manifest 18 months (≤ 2 years) after the index surgery. As Zappe *et al.* (2008) mentioned in their publication *Propionibacterium* spp. is mainly associated with low-grade infections which typically present with subtle signs and often develop within 24 months of implantation. However, neither CRP nor ESR values were helpful in establishing or excluding the diagnosis of *P. acnes* associated PJI in their report. The parameters of systemic markers of inflammation also remained within the normal range in our patient; however, as it has been mentioned above, the cytological analysis of the joint fluid was indicative of an ongoing infectious process which enabled to ascribe an important pathogenic role to both isolated bacteria: *S. epidermidis* and *P. acnes*. Similarly to our observations, co-infection with *P. acnes* and CNS was reported by Zappe *et al.* (2008) and these bacteria were considered true pathogens in their study as well. These authors, however, based the diagnosis of PJIs on the results of culture of synovial fluid and periprosthetic tissues; sonication was not performed.

P. acnes was detected in only one patient in our study. The overwhelming majority of bacteria cultu-

red from patients (irrespective of the initial clinical classification of the loosening) were CNS which is in agreement with results reported by other researchers (Perdreau-Remington *et al.*, 1996; Trampuz *et al.*, 2007; Schäfer *et al.*, 2008; Achermann *et al.*, 2010). It should be mentioned that CNS generally account for the majority of causative agents of PJIs (30–43%), followed by streptococci (9–10%), enterococci (3–7%), Gram-negative bacilli (3–6%) and anaerobes (2–4%). The frequency of *S. aureus* associated PJIs is 12–23% (Trampuz and Zimmerli 2005, Zimmerli and Moser 2012). It is worth mentioning that patients with the clinical diagnosis of PJI in our study were more frequently infected with *S. aureus* (three patients) than patients whose loosening was initially classified as aseptic (one patient). Microorganisms other than staphylococci (and the above mentioned *P. acnes*) such as *E. cloacae* and *S. mitis* were cultivated from single patients in our study. These patients had a clinical diagnosis of aseptic implant failure.

It is interesting to note that virulent bacteria such as *S. aureus* or Gram-negative rods are usually responsible for early infections (< 3 months after implantation (Zimmerli *et al.*, 2004; Trampuz and Zimmerli, 2005) but it was the case for only one patient infected with *S. aureus* in our study. In the remaining patients time interval between surgery and the onset of loosening symptoms was 14–28 months (*S. aureus)* and 169 months (*E. cloacae*) suggesting the delayed and late infections. Delayed infections are diagnosed within 3–24 months after surgery, whereas infections diagnosed beyond this period are defined as late PJI (Butler-Wu *et al.*, 2011). The lack of clinical symptoms of an ongoing infectious process among two patients infected with *S. aureus* and *E. cloaceae* was surprising taking into account the significant pathogenic potential of these bacteria and their isolation from multiple clinical samples. Our observations also indicate that both the implant surface and, in consequence, the neighbouring tissues may become infected throughout the period of functioning of the prosthesis in the body as late infections are predominantly caused by haematogenous seeding of microorganisms from remote foci of infection (Zimmerli and Moser 2012).

It should be mentioned that the sonication technique applied in our study was burdened with some limitations. Namely, the sonicate-fluid cultures were negative in three patients with overt manifestations of PJIs and positive tissue culture results. There are several possible explanations of ineffectiveness of sonication in terms of isolation of etiologic agents of implant dysfunction. Sonication can be insufficient to remove bacteria strongly adherent to the device. In addition, in some cases the microorganisms may predominantly colonize bone rather than the prosthesis surface. Furthermore, implants may be infected with viable but nonculturable bacteria which may be a result of prolonged starvation associated with depletion of nutrients, accumulation of waste products and high bacterial density within the deep layers of the biofilm structure (Tunney *et al.*, 1999; Fux *et al.*, 2005). Finally, bacteria from implants may also be sensitive to mild ultrasonication due to stress associated with prosthesis removal and transportation to the laboratory for processing. It is also conceivable that viable but nonculturable bacteria are so highly adapted to the environment of the *in vivo* biofilm that the conditions required for their continued growth and isolation are not met by the growth media and isolation procedures applied. Moreover, dilution of bacterial quorum sensing system signaling molecules, of which a critical concentration may be necessary to trigger microbial growth, may account for difficulties in their isolation. In other words, bacteria growing for long periods in a relatively static environment, largely protected from the immune system, may, in the long term, become less adaptable and more difficult to cultivate (Tunney *et al.*, 1999).

It can be concluded that the diagnostic approach towards PJIs should include both sonication and conventional culture of periprosthetic tissue fragments and the intraoperative synovial fluid samples. The combination of various cultivation techniques increases the chance of successful isolation and identification of microorganisms involved in prosthesis dysfunction.

Negative culture results are generally reported in case of 7–11% of apparent PJIs (Zimmerli *et al.*, 2004, Esposito and Leone 2008). We obtained negative culture results in 4 (18%) out of 22 patients who developed a sinus tract indicative of PJI in spite of a combination of sonication and tissue cultures. Similar problems were reported by Trampuz *et al.* (2007). Among 79 patients with PJI enrolled in their study negative culture results were reported for 17 patients. These discrepancies were explained by the authors as the possibility of the presence of unusual microbes which were unable to grow under conditions provided, due to the use of inappropriate media, inadequate incubation time, the loss of viability during transport of the specimen or the previous or prophylactic antimicrobial therapy.

Finally, the application of a multidirectional diagnostic approach including the results of microbiological culture and the analysis of the levels of local and systemic inflammatory mediators led us to assumption of the aseptic loosening as the most probable cause of implant dysfunction (constituting a confirmation of the initial clinical diagnosis) in 43 out of the 54 patients. In spite of limitations of cultivation techniques non-microbial pathogenesis of prosthetic joint loosening cannot be excluded and was also a subject of investigation conducted by Trampuz *et al.* (2007) and Ince *et al.* (2004).

To conclude, a combination of various cultivation techniques with particular emphasis on sonication should be used in order to overcome diagnostic problems standing behind PJIs. Sonication was characterized by the highest sensitivity in our study. It appears a particularly valuable diagnostic tool in patients whose dysfunctional prostheses are assumed to have aseptic nature due to the lack of clinical manifestations of an ongoing infection. Nevertheless, culture-based methods (which carry a risk of both false-positive and false-negative results) should be interpreted in the aspect of the levels of local and systemic inflammatory mediators. Among them synovial fluid leukocyte count and the differential hold a particular promise as early predictors of PJIs.

Conflict of Interest

The authors declare that they have no conflict of interest.

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